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Comparison of the Insecticidal Characteristics of Commercially Available Plant Essential Oils Against Aedes aegypti and Anopheles gambiae (Diptera: Culicidae)

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Abstract
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Keywords
Aedes aegypti, Anopheles gambiae, plant essential oil, synthetic pyrethroid, terpene

Disciplines
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Comments
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Comparison of the Insecticidal Characteristics of Commercially Available Plant Essential Oils Against *Aedes aegypti* and *Anopheles gambiae* (Diptera: Culicidae)

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ABSTRACT *Aedes aegypti* and *Anopheles gambiae* are two mosquito species that represent significant threats to global public health as vectors of Dengue virus and malaria parasites, respectively. Although mosquito populations have been effectively controlled through the use of synthetic insecticides, the emergence of widespread insecticide-resistance in wild mosquito populations is a strong motivation to explore new insecticidal chemistries. For these studies, *Ae. aegypti* and *An. gambiae* were treated with commercially available plant essential oils via topical application. The relative toxicity of each essential oil was determined, as measured by the 24-h LD_{50} and percentage knockdown at 1 h, as compared with a variety of synthetic pyrethroids. For *Ae. aegypti*, the most toxic essential oil (patchouli oil) was ~1,700-times less toxic than the least toxic synthetic pyrethroid, bifenthrin. For *An. gambiae*, the most toxic essential oil (patchouli oil) was ~685-times less toxic than the least toxic synthetic pyrethroid. A wide variety of toxicities were observed among the essential oils screened. Also, plant essential oils were analyzed via gas chromatography/mass spectrometry (GC/MS) to identify the major components in each of the samples screened in this study. While the toxicities of these plant essential oils were demonstrated to be lower than those of the synthetic pyrethroids tested, the large amount of GC/MS data and bioactivity data for each essential oil presented in this study will serve as a valuable resource for future studies exploring the insecticidal quality of plant essential oils.

KEY WORDS *Aedes aegypti*, *Anopheles gambiae*, plant essential oil, synthetic pyrethroid, terpene

Of all the insect families, Culicidae poses the greatest threat to human public health throughout the world (Service 2012). Although most mosquito species are nuisance species that do not vector disease agents, many transmit organisms that cause some of the deadliest and most debilitating diseases known to both humans and domestic animals. Dengue fever, yellow fever, lymphatic filariasis, and malaria are just a few of the many diseases caused by the etiologic agents vectored by various mosquito species (Service 2012). In 2012, the World Health Organization (WHO) estimated that >207 million people were infected with malaria parasites, resulting in the loss of ~627,000 lives, many of whom were children in sub-Saharan Africa and Southeast Asia (WHO 2013). Unfortunately, the actual number of deaths could be much higher, as many cases of malaria in developing countries go unreported (WHO 2013).

With the advent of insecticide-resistant mosquito populations, the risk of mosquito-borne disease epidemics is even greater than in previous decades (WHO 1970). Since the widespread use of DDT in the late 1940s and early 1950s, mosquito populations throughout the world have been steadily developing resistance to various classes of insecticides (Pampana and Russell 1955, Pampana 1963, Hemingway and Ranson 2000). Mosquitoes have acquired resistance to organochlorines, organophosphates, and some synthetic pyrethroids through multiple molecular and biochemical adaptations. Mutations in genes that encode enzymes and other proteins that are targeted by various insecticidal classes can diminish the interaction between insecticides and these proteins, limiting their overall effectiveness (Oppenoorth 1984, Davies et al. 2008, Ffrench-Constant 2013). Also, up-regulation of or mutations in genes that encode detoxification enzymes can also confer resistance by enabling insect pest species to more effectively metabolize or remove xenobiotics from their cells and tissues (Grant and Hammock 1992, Feyereisen et al. 1995, Berge et al. 1998). Currently, synthetic pyrethroids are the most widely used class of insecticides for controlling mosquito

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populations. Unfortunately, mosquito populations that are resistant to many synthetic pyrethroids have already been reported, and more will undeniably be identified in the coming decades due to the repeated application and overuse of this insecticidal class (Santolamazza et al. 2008, Hardstone et al. 2009, Ranson et al. 2011).

Synthetic pyrethroids were designed after natural pyrethrins, insecticidal compounds isolated from *Chrysanthamum cinerariifolium* (Tattersfield et al. 1929, Mclaughlin 1973, Casida 1980, Ruigt 1985). Although the naturally occurring compounds are quite insecticidal in the natural form, they possess virtually no residual activity in the environment. A broader spectrum of activity against a large array of arthropod species and improved photostability were further developed by synthesizing analogs with aromatic rings and halogens (Elliot et al. 1973, Elliot and Janes 1978; Elliot 1980). Indeed, many synthetic bioactive compounds on the market today were designed after naturally occurring compounds. The gamut of compounds produced by bacteria, fungi, plants, and animals represent large repositories that could be tapped for the pursuit of creating novel insecticides. The variety of components within many commercial plant essential oils is an example of such a repository.

Plant essential oils are composed of hydrophobic, volatile compounds that are separated from the vegetative parts of plants by means of steam distillation or solvent extraction. Their defining quality is that they possess the same aroma, or essence, of the plant from which they are extracted (Cheng et al. 2003, Amorati et al. 2013). These oils are primarily composed of terpenoids and phenyl propanoids, which are biosynthetically produced by plants through either the isoprenoid biosynthesis or shikimate pathway (Sangwan et al. 2001). Some terpenoids repel or kill various arthropod pests and have also been implicated in the attraction of pollinators and other beneficial species. For example, some extracts from amyris (*Amyris balsamifera*) and Siamwood (*Fokienia hodginsii*) have been implicated in the repellency of mosquito species, while volatiles from *Brassica oleracea* have been implicated in the attraction of various parasitoids that prey on caterpillars that damage the plants (Paluch et al. 2009, Maia and Moore 2011, Poelman et al. 2012, Harrewijn et al. 1994). While the chemistry of many plant essential oils has been well documented, there is still much to learn about their respective bioactivities, particularly in regards to repellent or lethal activity against insects.

To date, the understanding of plant essential oil mode of action is diverse and complex as multiple studies suggests that many molecular targets are involved. In *Drosophila melanogaster*, the binding affinities of select terpenoids to a heterologously expressed tyramine receptor correlate directly with the toxicity of these terpenoids in the wild-type insect (Essam 2005). Also, significant specific binding of various terpenoids to the *Periplaneta americana* octopamine receptor further suggests that some of these terpenoids may be bioactive at these sites (Essam 2001). Plant essential oil components also exert their effect through many other modes of action, for example, by binding to $GABA_A$ receptor ion channel agonists, as acetylcholinesterase inhibitors, and as nicotinic acetylcholine receptor agonists (Tong and Coats 2010, Anderson and Coats 2012, Tong et al. 2012). Because of their diverse modes of action, which are unique to many of the insecticidal compounds on the market today, there is minimal likelihood of cross-resistance with currently available insecticides. Plant essential oils and their components may prove to be valuable tools in the pest management arsenal.

For this study, we screened a wide array of commercially available essential oils for toxicity and knockdown activity against the yellow fever mosquito, *Aedes aegypti*, and the African malaria mosquito, *Anopheles gambiae*. The essential oils in this study were chosen to represent a large diversity of potential chemistries from different plant genera and for their commercial availability. From these data, we generated LD_{50} values to compare the effects of these oils both within and between species. We also recorded another potential metric of insecticidal action, knockdown (KD) at 1 h. The data illustrates the potential of whole plant essential oils to control adult female mosquitoes and identifies essential oils that may possess compounds that could prove to be insecticidal in future studies.

Materials and Methods

Mosquitoes. *Aedes aegypti.* Adults were housed in colony cages (47 by 47by 47 cm^3) and reared at 27°C and 70% relative humidity. A 10% sucrose solution was supplied ad libitum via a saturated cotton pad. Mosquitoes were blood-fed regularly to promote egg laying. Defibrinated sheep's blood (Hemostat Laboratories, Dixon, CA) was supplied as a blood source via an artificial membrane feeding system. Eggs were collected from cages 4 d after blood-feeding and were stored until needed for hatching. Eggs were hatched in a pan of deionized water, and larvae were supplied different amounts of TetraMin Tropical Flakes Fish Food (Tetra, Blacksburg, VA), based on larval instar and density. After treatment, adults were supplied a 10% sucrose solution ad libitum via saturated cotton pads.

Male and female pupae were separated based on distinct differences in size (females are larger) via an upright separator. Adults were kept in 1-pt. cartons (Huhtamaki, De Soto, KS) in densities of 50 per carton. Adults in cartons were fed 10% sucrose solution in a saturated cotton ball placed atop the netting. Cotton balls were remoistened daily.

Anopheles gambiae. The protocol for rearing mosquitoes of this species was similar to that of *Ae. aegypti*; however, vinyl sheeting was wrapped around cages to maintain a higher relative humidity. The blood source for *An. gambiae* adults was primarily a live rabbit (*Oryctolagus cuniculus*), but defibrinated sheep's blood (Hemostat Laboratories, Dixon, CA) was occasionally used.

Because no profound size sexual dimorphism exists between males and females of this mosquito species, males and females were separated by aspirators shortly after emergence. After emergence, females were

introduced into new cups at the same concentration (50 mosquitoes per cup) as *Ae. aegypti.*

Essential oils/synthetic pyrethroids. Synthetic pyrethroids were obtained from a variety of sources. Permethrin *Z:E* 40:60 (purity 98%) and bifenthrin (purity 97%) were obtained from EcoSMART Technologies Inc., Roswell, GA. β-cyfluthrin (purity 99.8%) and deltamethrin (purity 99.7%) were obtained from Sigma-Aldrich Co. LLC., St. Louis, MO. λ-Cyhalothrin (purity 97.1%) was obtained from Controlled Solutions Inc., Pasedena, TX. Essential oils were supplied by EcoSMART Technologies Inc. and were originally obtained from Berjé Inc., Carteret, NJ. To limit variability within oil samples, lot numbers were associated with each essential oil. In the case of resupplying, identical batches of essential oils were delivered for the entirety of the project. Gas chromatography/mass spectrometry (GC/MS) analysis for each essential oil enabled the identification of the predominant components within each essential oil. All solutions used for topical application were prepared in Certified ACS grade acetone (Thermo Fisher Scientific, Waltham, MA).

Topical Application. Topical applications on adult, female mosquitoes were performed using a modified WHO protocol (World Health Organization Pesticide Evaluation Scheme [WHOPES] 2006). Essential oils and synthetic pyrethroids were dissolved in certified acetone at various concentrations that would yield between 5% and 95% mortality at 24 h posttreatment. Mosquitoes were anesthetized with carbon dioxide and quickly transferred to a Petri dish surrounded by ice to prevent reanimation. A filter paper was placed at the bottom of the Petri dish to absorb condensation and replaced with a new filter paper for each new compound tested. For each application, a 0.2-μl volume of solution was applied to the pronotum of each female mosquito using a 10-μl gastight Hamilton syringe, and treated mosquitoes were transferred to a 4-ounce cup with tulle placed on the top to prevent escape. Topical applications took ~2–3 min to complete for each concentration of each essential oil (25 mosquitoes total). The time at which the last treated female mosquito was placed in the cup was recorded and used as the dosage time for the 1-h percentage knockdown and 24-h percentage mortality readings. Treated mosquitoes were then moved to an environmentally controlled incubator (27°C, 80% relative humidity, and a photoperiod of 16:8 [L:D] h) for 24 h, at which point mortality was recorded. Mortality at 24 h was defined as the percentage of insects that showed no movement (ataxia) after being prodded with a camel hair brush. The same procedures were followed for the 1-h knockdown studies; however, observations were recorded at 1-h as opposed to at 24 h. Knockdown (KD) was defined as the inability of a mosquito to fly or orient itself in the upright direction and was recorded at 1 h postapplication.

To assess the LD_{50} for each compound or essential oil, data were collected for at least five concentrations that yielded between 5% and 95% mortality at 24 h. In total, 25 mosquitoes were treated per replicate, and a minimum of three replicates (25 mosquitoes per replicate) from different rearing cohorts were conducted for each concentration. "Acetone only" controls were conducted every day (sample size/synthetic pyrethroid or essential oil ≥375 mosquitoes). Data were not used for analysis if 24-h mortality was >10% in the control. Because *Ae. aegypti* females weighed nearly twice as much as the female *An. gambiae* females, all toxicity data are reported in microgram of insecticide per gram of body weight.

Data Analysis. Mortality data were analyzed via the log-probit method described by Finney (1971) by using the Probit software (PROC PROBIT, SAS Institute Inc. 2012, Cary, NC) with the option to account for the control response (OPTC command). More replicates were performed if the probability > chi-squared test parameter (Pr > was > 0.05. One-hour knockdown percentages within each oil were compared with 24-h mortality percentages for that oil using a *t*-test (PROC TTEST, SAS Institute Inc. 2012) with the assumption of equal variance to detect 1-h knockdown percentages that were statistically higher than 24-h mortality percentages at a significance level of 0.05 ($\alpha = 0.05$).

Results

LD_{50} Results. Within *Ae. aegypti*, a 27-fold range of LD_{50} values was observed among the essential oils screened (patchouli oil = 1,500 μg/g to sassafras oil = 40,400 μg/g; Table 1). Among the synthetic pyrethroids, a 31-fold range of LD_{50} values was demonstrated (β-cyfluthrin = 0.028 μg/g to bifentrhin = 0.87 μg/g; Table 2). The most toxic synthetic pyrethroid tested was ~ 50,000-times more potent compared to the most toxic essential oil (Tables 1 and 2). The least toxic synthetic pyrethroid, bifenthrin, was ~ 1,700-times more potent than the most toxic essential oil, patchouli oil (Tables 1 and 2).

Within *An. gambiae*, there was a 62-fold range of LD_{50} values among the essential oils screened (patchouli oil = 500 μg/g to rosemary oil = 31,000 μg/g; Table 3). Among the synthetic pyrethroids, there was a 243-fold range in LD_{50} values (deltamethrin = 0.003 μg/g to bifenthrin = 0.73 μg/g; Table 2). The most toxic synthetic pyrethroid, deltamethrin, for this species was ~167,000-times more potent than the most toxic essential oil, patchouli oil. The least toxic synthetic pyrethroid, bifenthrin, was ~685-times more potent than the most toxic essential oil, patchouli oil.

Between the two species, *An. gambiae* appeared to be more susceptible to both the essential oils and synthetic pyrethroids than *Ae. aegypti* (Tables 1 and 3). For the essential oils, there was up to a 16-fold disparity between the LD_{50} values, as demonstrated by those obtained for each species for catnip oil, with *An. gambiae* being much more susceptible to this essential oil. However, there were exceptions to this general trend with *Litsea cubeba*, cedar leaf, and basil oil all being less toxic to *An. gambiae* than to *Ae. aegypti.* Also, within each species, there was variation in the essential oils. For instance, catnip, amyris, and guaiacwood oil were all more toxic to *An. gambiae*, compared with the other essential oils. These oils were considerably less

Table 1. Susceptibility of adult female *Ae. aegypti* to a variety of commercially available plant essential oils

Treatment	*n*	Slope (SE)	LD_{50}[a]	95% FL[a]	χ^2(df)[b]
Patchouli	1,100	1.62 (.31)	1,500	1,000–2,000	242.6 (42)
Cassia	750	2.35 (0.46)	3,300	2,700–4,100	108.83 (28)
Thyme	425	4.90 (0.84)	3,400	3,000–4,000	32.55 (15)
Litsea cubeba	625	5.84 (1.01)	3,400	3,000–4,000	65.26 (23)
Origanum	600	5.25 (0.57)	3,500	3,000–4,000	38.98 (22)
Cinnamon leaf	825	5.64 (0.70)	3,500	3,300–3,800	75.9 (31)
Sandalwood (Australian)	525	3.04 (0.92)	3,600	3,000–5,000	145.2 (19)
Cinnamon bark	475	5.42 (0.98)	3,700	3,000–4,000	70.21 (17)
Clove bud	622	3.59 (0.63)	4,100	3,000–5,000	114.6 (23)
Clove leaf	575	5.98 (0.77)	4,200	3,800–4,500	61.52 (21)
Citronella (Java)	775	3.88 (0.60)	4,500	4,000–5,000	105.7 (29)
Lemongrass	375	7.08 (1.14)	4,900	4,000–5,000	23.73 (13)
Geranium (Bourbon)	575	5.05 (0.70)	6,000	5,000–7,000	69 (21)
Catnip	375	5.57 (2.02)	9,000	8,000–10,000	42.6 (13)
Amyris	400	3.51 (0.47)	9,400	8,000–11,000	72.7 (26)
Cedarwood (Texas)	500	7.90 (1.67)	10,700	9,000–12,000	65.81 (18)
Cedar leaf	500	5.59 (1.14)	10,500	9,000–12,000	73.9 (18)
Guaiacwood	450	4.36 (0.68)	10,500	10,000–12,000	26.5 (16)
Basil (Egyptian)	775	5.5 (0.65)	10,900	10,000–12,000	78.3 (29)
Anise seed	500	3.62 (0.5)	11,600	11,000–13,000	26.6 (18)
Peppermint (*M. piperita*)	525	4.29 (0.68)	12,700	11,000–14,000	46.98 (19)
Cedarwood (Moroccan)	725	7.50 (1.53)	12,700	11,000–15,000	157.3 (27)
Celery seed	450	3.57 (0.49)	14,600	13,000–16,000	21.41 (15)
Sesame	600	4.58 (2.87)	15,000	12,000–18,000	136.7 (22)
Nutmeg (West Indies)	550	6.09 (1.04)	19,100	18,000–21,000	56.71 (20)
Wormwood (American)	550	6.11 (0.80)	20,200	18,000–22,000	44.9 (20)
Orange	950	6.00 (0.99)	22,500	19,000–27,000	177.8 (39)
Parsley seed	475	4.44 (0.64)	24,000	22,000–25,000	19.24 (17)
Black pepper	475	6.59 (1.3)	31,500	30,000–34,000	49.3 (17)
Rosemary	400	8.64 (2.09)	33,000	31,000–35,000	29.58 (14)
Nutmeg (East Indies)	485	5.75 (1.3)	33,300	31,000–36,000	20.9 (17)
Wintergreen	500	4.29 (0.56)	39,700	37,000–42,000	20.09 (19)
Sassafras	400	2.77 (0.64)	40,400	36,000–46,000	19.51 (14)

[a] All LD_{50} values were calculated using an average weight of 2.54 mg per female mosquito ($n = 256$ mosquitoes).
[b] Pearson's chi-square goodness-of-fit values with degrees of freedom (df). Degrees of freedom are used to calculate significance in the model at a threshold of $P < 0.05$.

toxic relative to the other essential oils when screened against *Ae. aegypti*. (Tables 1 and 3). This general trend was also true for the synthetic pyrethroids. The disparity in the LD_{50} values for these compounds was much greater than that observed for the oils between species, with deltamethrin having a ~193-fold greater effect against *An. gambiae* than *Ae. aegypti*. Again, there were exceptions to the trend among the synthetic pyrethroids, with *Ae. aegypti* being more susceptible to permethrin than *An. gambiae* (Table 2).

There were also some major differences in the toxicities of essential oils between species. For example, clove leaf and clove bud oils possessed different LD_{50} values against *An. gambiae*, with clove leaf being about twice as toxic than clove bud (Table 3). For *Ae. aegypti*, nutmeg (West Indies) oil was more toxic than nutmeg (East Indies) oil (Table1). The large percentage of α- and β-pinene in the nutmeg (West Indies) oil could explain the greater toxicity of this oil compared with nutmeg (East Indies) oil (Supp Table 1 [online only]). The opposite was true for *An. gambiae* (Table 3). Another example of a stark difference in relative toxicity among the oils was *L. cubeba*. While it was the 4th most toxic essential oil for *Ae. aegypti*, this essential oil was only the 17th most toxic essential oil for *An. gambiae*. Catnip also possessed marked differences in toxicity between the two species. While possessing relatively high toxicity for *An. gambiae* ($LD_{50} = 600$ μg/g), it was only one of the moderately toxic oils for *Ae. aegypti* ($LD_{50} = 9{,}000$ μg/g). Cedar leaf oil also demonstrated a major relative toxicity difference between species, being the 17th most toxic essential oil against *Ae. aegypti* and the 30th most toxic essential oil against *An. gambiae*. This was also true for basil (Egyptian) oil, as it was the 19th most potent essential oil against *Ae. aegypti* and only the 31st most toxic essential oil for *An. gambiae*.

One-Hour Knockdown Results. Concentrations of essential oils were chosen to ensure between 5% and 95% mortality at 24 h. Because of the wide range of toxicities and concentrations used for all of the essential oils, it was impossible to compare all essential oils at a single dose within species that would cause measurable 24-h percentage mortality and 1-h percentage knockdown. To compare the essential oils, they were organized into separate groups that enabled the comparison at particular concentrations within each group.

For *Ae. aegypti*, three concentrations (6, 15, and 40 μg) were tested that corresponded to groups of essential oils demonstrating three levels of toxicity: most, moderately, and least toxic, respectively (Fig. 1). For many of the essential oils, the 1-h knockdown and 24-h mortality values were similar at the concentrations

Table 2. Susceptibility of adult female *An. gambiae* to a variety of commercially available plant essential oils

Treatment	*n*	Slope (SE)	LD_{50}[a] (μg/g mosquito)	95% FL[a] (μg/g mosquito)	χ^2 (df)[b]
Patchouli	925	3.95 (0.65)	500	450–600	45.87 (27)
Catnip	500	3.12 (0.49)	600	500–700	31.93 (14)
Sandalwood (Australian)	625	2.86 (0.43)	1,300	800–1,700	36.56 (20)
Clove leaf	825	3.21 (0.53)	1,500	1,200–1,800	96.16 (26)
Cassia	675	4.34 (0.88)	1,500	1,300–1,800	39.21 (19)
Origanum	575	3.15 (0.5)	1,600	1,300–2,000	42.04 (17)
Thyme	650	3.64 (0.82)	1,700	1,100–2,200	77.02 (18)
Cinnamon Bark	750	3.46 (0.85)	2,100	1,600–2,700	150.65 (24)
Amyris	925	3.513 (0.47)	2,100	1,800–2,400	72.69 (26)
Guaiacwood	500	3.69 (0.38)	2,500	2,200–2,700	21.38 (15)
Geranium (Bourbon)	550	3.28 (0.73)	2,600	1,800–3,300	41.8 (15)
Cinnamon Leaf	825	4.14 (0.91)	2,900	2,400–3,500	101.02 (26)
Lemongrass	800	3.69 (0.70)	3,000	2,200–3,700	71.82 (23)
Clove bud	500	3.45 (0.41)	3,200	2,800–3,700	24.07 (14)
Cedarwood (Texas)	825	3.45 (0.42)	3,800	3,300–4,300	45.65 (25)
Citronella	1,075	3.91 (0.71)	3,900	3,500–4,400	128.05 (33)
L. cubeba	800	6.64 (1.20)	4,000	3,500–4,400	72.29 (25)
Anise	825	1.99 (0.70)	4,500	2,400–8,600	205.94 (26)
Parsley Seed	700	1.99 (0.34)	5,000	3,500–6,600	62.57 (20)
Sesame	625	3.26 (0.53)	5,900	4,800–7,100	40.44 (18)
Celery Seed	775	3.14 (0.45)	6,600	5,500–7,900	68.19 (22)
Peppermint	775	4.06 (0.65)	6,800	5,400–8,100	84.99 (24)
Cedarwood (Moroccan)	575	5.15 (1.25)	7,700	6,600–9,400	41.82 (16)
Black Pepper	575	4.91 (1.70)	8,000	6,500–10,000	54.79 (21)
Sassafras	525	2.41 (0.37)	10,000	7,700–13,000	28.96 (15)
Nutmeg (East Indies)	600	3.32 (0.32)	10,500	9,300–12,000	13.87 (16)
Wintergreen	825	2.84 (0.47)	11,100	9,200–13,000	60.25 (24)
Orange	725	1.94 (0.67)	11,100	1000–18,000	87.81 (22)
Wormwood	600	4.64 (0.74)	12,000	10,000–13,000	26.5 (18)
Cedar leaf	500	4.49 (0.87)	15,000	13,000–17,000	33.95 (15)
Basil (Egyptian)	725	3.62 (0.78)	18,000	15,000–22,000	54.79 (21)
Nutmeg (West Indies)	850	3.66 (0.82)	19,000	15,000–22,000	76.54 (25)
Rosemary	450	8.49 (1.66)	31,000	26,000–34,000	14.38 (13)

[a] All LD_{50} values were calculated using an average weight of 1.36 mg per female mosquito for *An. gambiae* ($n = 318$ mosquitoes).
[b] Pearson's chi-square goodness-of-fit values with degrees of freedom (df). Degrees of freedom are used to calculate significance in the model at a threshold of $P < 0.05$.

Table 3. Susceptibility of adult female *Ae. aegypti* and *An. gambiae* to a variety of synthetic pyrethroid insecticides

Species	Treatment	*n*	Slope (SE)	LD_{50}[a] (μg/g mosquito)	95%FL[a] (μg/g mosquito)	χ^2 (df)[b]
Aedes aegypti	λ-cyhalothrin	1025	0.93 (0.39)	0.061	0.03–453.86	178 (31)
Aedes aegypti	λ-cyhalothrin	1100	1.16 (0.33)	0.03	0.01–0.05	136.5 (33)
Aedes aegypti	β-cyfluthrin	1575	0.84 (0.15)	0.028	0.018–0.058	199.57 (51)
Anopheles gambiae	β-cyfluthrin	1425	1.29 (0.19)	0.035	0.03–0.04	75.07 (44)
Aedes aegypti	Deltamethrin	1525	0.39 (0.08)	0.58	0.21–5.16	120.03 (50)
Anopheles gambiae	Deltamethrin	850	1.15 (0.2)	0.003	0.001–0.004	88.24 (28)
Aedes aegypti	Bifenthrin	750	2.51 (0.4)	0.87	0.71–1.04	50.7 (24)
Anopheles gambiae	Bifenthrin	725	2.39 (0.34)	0.73	0.57–0.89	45.96 (23)
Aedes aegypti	Permethrin	1300	1.93 (0.3)	0.41	0.3–0.51	129.65 (23)
Anopheles gambiae	Permethrin	1350	1.55 (0.17)	0.63	0.48–0.8	115.31 (44)

[a] All LD50 values were calculated using an average weight of 2.54 mg per female mosquito for *Ae. aegypti* ($n = 256$ mosquitoes) and 1.36 mg per female mosquito for *An. gambiae* ($n = 318$ mosquitoes).
[b] Pearson's chi-square goodness-of-fit values with degrees of freedom (df). Degrees of freedom are used to calculate significance in the model at a threshold of $P < 0.05$.

tested. Listing these oils from most toxic to least, patchouli, thyme, cinnamon leaf, clove bud, clove leaf, catnip, amyris, guaiacwood, celery seed, nutmeg East Indies, and sassafras essential oils all exhibited 1-h knockdown percentages that were considerably greater than the 24-h mortality percentages observed at each respective screening concentration. Of these, patchouli ($94 \pm 2\%$ KD vs. $24 \pm 4\%$ mortality), origanum ($2 \pm 2\%$ KD vs. $22 \pm 2\%$ mortality), cinnamon leaf ($60 \pm 9.7\%$ KD vs. $11 \pm 3.8\%$ mortality), clove bud ($64 \pm 20\%$ KD vs. $2 \pm 2\%$ mortality), clove leaf ($74.7 \pm 6.7\%$ KD vs. $2.67 \pm 1.3\%$ mortality), guaiacwood ($69.3 \pm 6.7\%$ KD vs. $10.7 \pm 1.3\%$ mortality), and celery seed oil have 1-h percentage knockdown that are

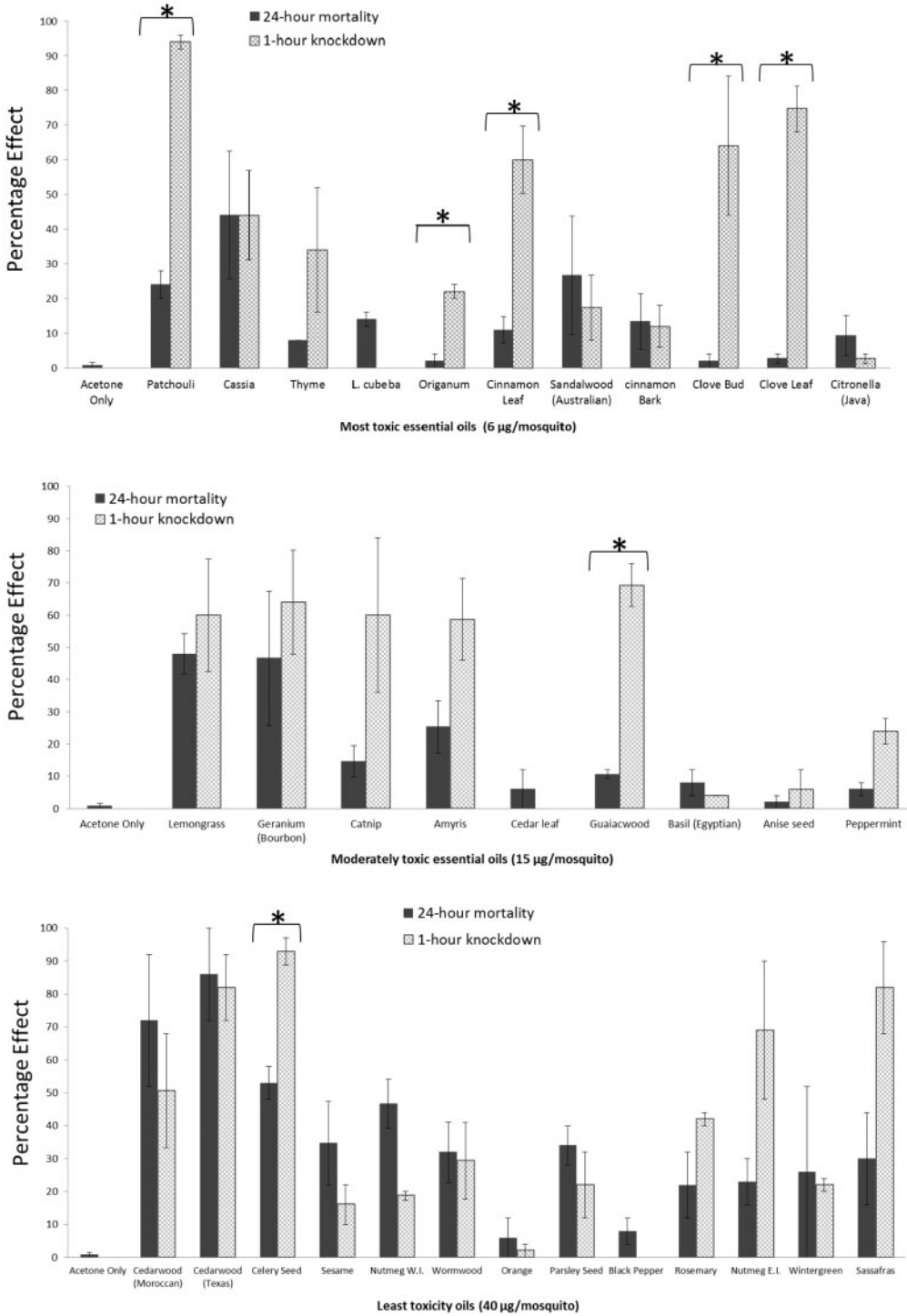

Fig. 1. The 24-hour percentage mortality and 1-hour percentage knockdown of *Aedes aegypti* caused by various commercially available plant essential oils. Plant essential oils are arbitrarily grouped into three groups of different toxicities. This grouping allowed essential oils to be compared to one another at identical concentrations. For many oils, the 1-hour knockdown percentages are significantly higher for multiple oils than the 24-hour mortality percentages.

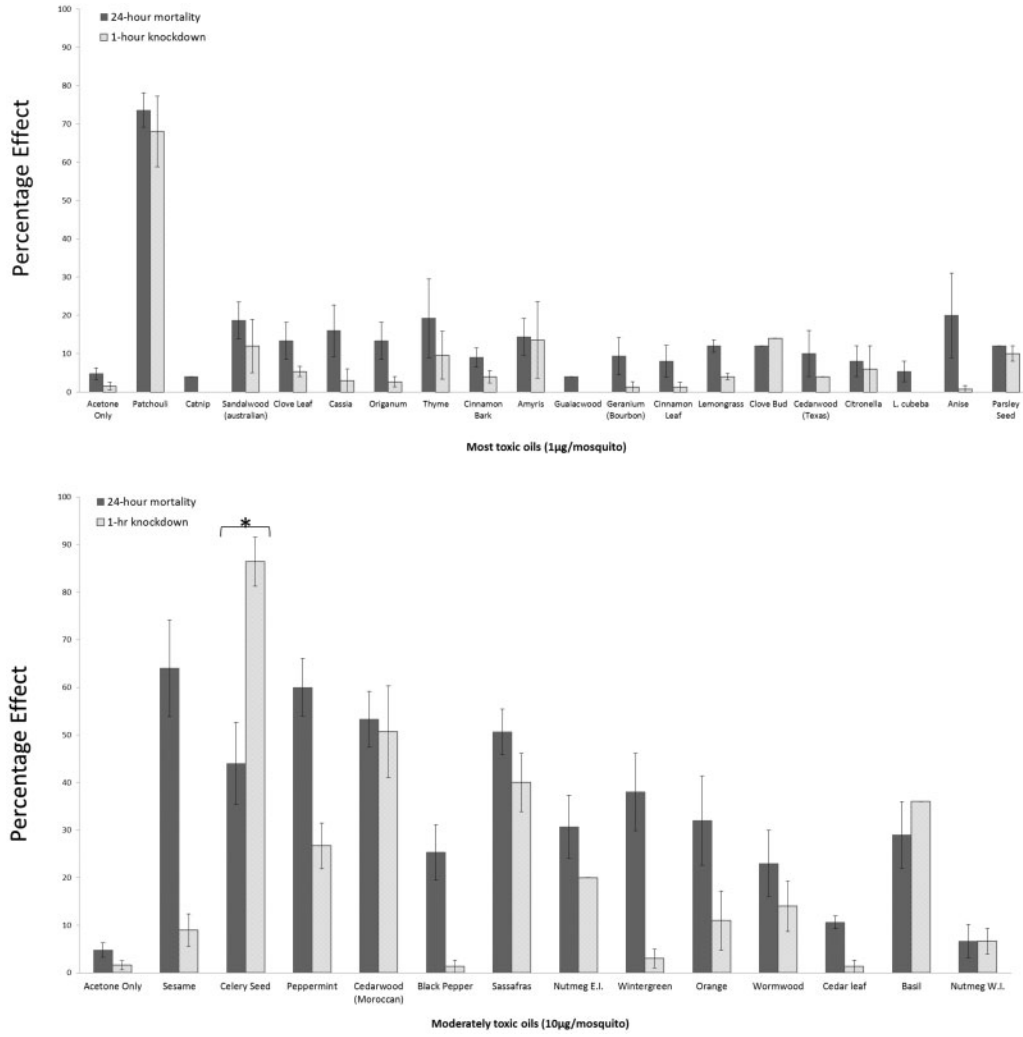

Fig. 2. The 24-hour percentage mortality and 1-hour percentage knockdown of *Anopheles gambiae* caused by various commercially available essential oils. Plant essential oils are arbitrarily grouped into two groups of different toxicities. This grouping allowed essential oils to be compared to one another at identical concentrations. Some oils caused significantly higher 1-hour knockdown percentages than 24-hour mortality percentages.

statistically greater than their respective percentage 24-h mortality.

The range of toxicities (and concentrations screened) of essential oils was narrower for *An. gambiae*. Two concentrations were chosen for comparisons corresponding to two groups: most toxic and moderately toxic essential oils, respectively. For *An. gambiae*, 1-h knockdown and 24-h mortality percentages at each respective concentration were more similar than for *Ae. aegypti* (Fig. 2). However, celery seed oil and basil oil caused higher 1-h knockdown percentages than mortality at 24 h. Of these two essential oils, only celery seed ($86.4 \pm 5.15\%$KD vs. $44 \pm 8.63\%$ mortality) oil demonstrated statistically significant higher percentage 1-h knockdown than its respective 24-h mortality percentage.

Discussion

The goal of this study was to explore a wide range of plant essential oils to determine whether or not plant essential oils could be used as effective insecticidal alternatives to other synthetic insecticides currently on the market. Although the LD_{50} values for any particular plant essential oil were much higher than all of the synthetic pyrethroids tested in this study, components within these plant essential oils (especially the most toxic) may prove to be effective insecticides toward adult female mosquitoes. For the sake of this discussion, essential oils were separated into three groups ranging from highest to lowest toxicities to draw general conclusions about the chemistries of components within each essential oil: most toxic (1–10 lowest LD_{50} values), moderately toxic (11–20 mid-range LD_{50}

values), and least toxic (21–33 highest LD_{50} values). The GC/MS data for this study are provided in Supp Table 1 (online only) and is alphabetized by each plant essential oil.

For both mosquito species, patchouli, cassia, thyme, origanum, cinnamon bark, clove leaf, and sandalwood oil all fell within the most toxic essential oil group for both species. Thyme, origanum, and clove leaf oil all contain large amounts of aromatic monoterpenoid (phenyl propanoid) compounds that have been documented as bioactive against various different arthropod species (Lee et al. 2003, Stamopoulos et al. 2007). Cassia oil and cinnamon bark oil both contain large quantities of cinnamaldehyde, a compound with insecticidal and bacteriocidal properties (Didry et al. 1994; Cheng et al. 2004, 2008). Patchouli and sandalwood oil contain large amounts of oxygenated sesquiterpenoids, such as E,Z-nuciferol, patchoulol, α- and β-santalol among others. While these compounds were implicated as antibacterial or antifungal in some studies (Vallejo et al. 2001, Lopes-Lutz et al. 2008), little is known about their bioactivity in arthropod systems.

Geranium (Bourbon) oil, lemongrass, citronella, and anise oil were all moderately toxic essential oils for both species. These essential oils possess a large amount of linear, oxygenated, or cyclic aliphatic monoterpenoid compounds: geraniol, menthol, citronellol, *trans*-verbenol, citronellal, and citral are the most predominant components of these oils. These essential oils have also demonstrated other bioactivity, such as spatial repellency to various species of mosquito (Moore et al. 2007).

Many of the oils possessed low to minimal toxicity for both species. For *Ae. aegypti*, the least toxic essential oil, sassafras oil, was ~46,000-times less toxic than the least toxic pyrethroid, bifenthrin. For *An. gambiae*, the least toxic essential oil, rosemary oil, was ~42,000-times less toxic than bifenthrin. Essential oils with low toxicity possessed lower amounts of aromatic monoterpenoids than their more toxic counterparts and were composed primarily of nonpolar hydrocarbons. It is possible that these compounds diffuse less rapidly through the aqueous hemocoel of the insect being treated and are therefore unable to exert any effect at various target tissues. Alternatively, these compounds may be easier to metabolize via detoxification enzymes or have less neurotoxic effects than the aromatic phenyl propanoids. However, there are exceptions to this. Myristicin (from nutmeg E.I. oil), cineole (from rosemary oil), thujone (from wormwood), menthol (from peppermint oil), and methyl salicylate (from wintergreen oil) are oxygenated components found in abundance in each of their respective oils and are significantly more polar than the other non-polar hydrocarbons.

Another metric that was utilized in this study to monitor insecticidal action was percentage knockdown at 1-h posttreatment (KD). Knockdown is a metric suggested by the WHO to determine the overall insecticidal characteristic of a compound. Some insecticide-resistant insect populations do not manifest the same level of knockdown as susceptible populations. Knockdown-resistance (kdr) mutations owe their name to this phenomenon (Briggs et al. 1974, Chang and Plapp 1983). It is possible that knockdown could be directly correlated to mortality in the field because of increased probability of desiccation or predation, by preventing the insect from obtaining water, escaping predators, or conducting grooming. This study demonstrates that the knockdown percentages for these essential oils are much higher than the 24-h mortality percentages for a number of essential oils. This knockdown effect suggests that some of these essential oils may act as effective insecticidal applications, despite causing a relatively low 24-h percentage mortality.

Moreover, the heightened 1-h percentage knockdown when compared with 24-h percentage mortality is particularly apparent in *Ae. aegypti*. In total, seven essential oils caused higher percentage knockdown at 1 h posttreatment than mortality at 24 h. This may suggest that *Ae. aegypti* have higher levels of detoxification enzymes that effectively rid the insect of toxic components from these oils. As previously shown by Chang and Plapp (1983), insects will experience knockdown initially after exposure to insecticides if they do not possess target site mutations conferring resistance. This suggests that recovery from an insecticidal challenge must be owing to detoxification enzymes. The lower toxicity of most of the essential oils and synthetic pyrethroids for *Ae. aegypti* when compared with *An. gambiae*, in general, may suggest different levels of detoxifying enzymes between the species.

While 24-h percentage mortality is extremely important in judging insecticidal efficacy, 1-h knockdown percentages may also translate to higher levels of mortality in the field. Knockdown in the field may contribute to mortality in a number of ways. By preventing adult females from obtaining nectar, it is possible that knockdown may contribute to desiccation or starvation. Also, adult mosquitoes are also more likely to be fed upon by predators if they are unable to escape. It has been demonstrated that insects use grooming behaviors for multiple reasons. Preventing the buildup of entomopathogenic fungi, which can lead to infections and death, is a primary function of this conserved behavior in many insects (Yanagawa et al. 2010). A high percentage 1-h knockdown may allow for entomopathogenic fungi to colonize adult female mosquitoes in the field, leading to high levels of mortality, even if the essential oil or components within do not cause high percentage mortality at 24 h.

This study illustrated that plant essential oils are demonstrably toxic to adult female mosquitoes. Although these plant essential oils may not be as toxic as synthetic insecticides used currently in the market, the may still be viable insecticidal agents by increasing the dose applied per insect, optimizing proper application rates, and changing formulation chemistry to effectively deliver these toxic oils or the individual terpenoids that they are comprised of. Plant essential oils may also be fairly variable in terms of their purity and availability. With the current essential oil market, plant essential oils under the same name may be sourced from multiple, potentially very distant geographic regions (Isman and Machial 2006). The variability in geographic region, soil, cultivation practices,

steam distillation processes and solar radiation at these disparate farm sites have been implicated in the differences in chemistry between plant essential oil batches (Djarri et al. 2008, Porter et al. 2010). The factors that contribute to this variability must be addressed if plant essential oils are to be used as future insecticides. Even with these hurdles, many companies today are marketing plant essential oil formulations as pesticides (Isman 2000).

Despite the drawbacks in plant essential oil production, plant essential oils are still promising potential insecticides for many reasons. As demonstrated through numerous studies, they exert their toxic effects through a wide array of modes of action, many of which are novel compared with synthetic insecticides on the market. This characteristic may be especially important in future insecticide resistance management regimens. By rotating between synthetic insecticides and plant essential oils or plant-derived compounds which affect different molecular targets within the insect, the implementation of plant essential oils in pest management programs may diminish the likelihood of insect populations developing resistance to synthetic insecticides. They may also be important in controlling insect populations that have already developed resistance to a large variety of synthetic chemistries, which tend to cause rapid inseciticide-resistance development.

This screening of a wide variety of commercially available plant essential oils accomplished multiple goals. By obtaining the LD_{50} values for various plant essential oils and comparing these data with those determined for various synthetic pyrethroids used heavily in mosquito control, we conclude that plant essential oils, overall, do not possess the same level of toxicity as synthetic pyrethroids. These plant essential oils are demonstrably insecticidal, especially the most efficacious oils screened. Furthermore, general conclusions were drawn about the chemistries of the different components of the most toxic, moderately toxic, and least toxic essential oil groups. This will enable further investigation into why these components are insecticidal, and through mode of action studies and quantitative structure–activity relationships, it may be possible to identify chemical derivitizations that create more toxic compounds. Finally, the different relative toxicities of plant essential oils to the two mosquito species, when paired with future mode of action studies, could lead to valuable insight into the susceptibilities and biology of each test organism. Although these plant essential oils did not possess the same level of toxicity toward these two mosquito species as synthetic pyrethroids, the components within these plant essential oils may still represent potential novel insecticidal compounds. The GC/MS data presented in this report for each of the essential oils tested will be a valuable reference for future studies that will isolate pure compounds to assess their respective bioactivities.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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References Cited

Amorati, R., M. Foti, and L. Valgimigli. 2013. Antioxidant activity of essential oils. J. Agric. Food Chem. 61: 10835–10847.

Anderson, J. A., and J. R. Coats. 2012. Acetylcholinesterase inhibition by nootkatone and carvacrol in arthropods. Pestic. Biochem. Physiol. 102: 124–128.

Berge, J. B., R. Feyereisen, and M. Amichot. 1998. Cytochrome p450 monooxygenases and insecticide resistance in insects. Philos. Trans. R. Soc. B. Biol. Sci. 353: 1701–1705.

Briggs, G. G., M. Elliott, A. W. Farnham, and N. F. Janes. 1974. Structural aspects of the knockdown of pyrethroids. Pest Manage. Sci. 5: 643–649.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environ. Health Perspect. 34: 189–202

Chang, C. P., and F. W. Plapp. 1983. DDT and pyrethroids: Receptor binding in relation to knockdown resistance (kdr) in the house fly. Pestic. Biochem. Physiol. 20: 86–91.

Cheng, S., J. Liu, C. Huang, Y. Hsui, W. Chen, and S. Chang. 2008. Insecticidal activities of leaf essential oils from Cinnamomum osmophloeum against three mosquito species. Bioresour. Technol. 100: 457–464.

Cheng, S., J. Liu, K. Tsai, W. Chen, and S. Chang. 2004. Chemical composition and mosquito larvacidal activity of essential oils from leaves of different Cinnamomom osmophloeum provenances. J. Agric. Food Chem. 52: 4395–4400.

Cheng, S. S., H. T. Chang, S. T. Chang, K. H. Tsai, and W. J. Chen. 2003. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. Bioresourc. Technol. 89: 99–102.

Davies, T.G.E., A. O. O'Reilly, L. M. Field, B. A. Wallace, and M. S. Williamson. 2008. Perspective: Knockdown resistance to DDT and pyrethroids: From target-site mutations to molecular modelling. Pest Manage. Sci. 64:1126–1130.

Didry, N., L. Dubreuli, and M. Pinkas. 1994. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm. Acta Helv. 69: 25–28.

Djarri, L., K. Medjroubi, S. Akkal, A. Elomri, E. Seguin, M. Groult, and P. Vérité. 2008. Variability of two essential oils of Kundmannia sicula (L.) DC., a traditional Algerian medicinal plant. Molecules 13: 812–817.

Elliott, M. 1980. Established pyrethroid insecticides. Pestic. Sci. 11: 119–28

Elliot, M., A. W. Farnham. N. F. Janes, P. H. Needham, and D. A. Pulman. 1973. A photostable pyrethroid. Nature 246: 169–170.

Elliott, M., and N. F. Janes. 1978. Synthetic pyrethroids–a new class of insecticide. Chem. Soc. Rev. 7: 473–505.

Essam, E. 2001. Insecticidal activity of essential oils: Octopaminergic sites of action. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 130: 325–337.

Essam, E. 2005. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Insect Biochem. Physiol. 59: 161–171.

Feyereisen, R., J. F. Andersen, F. A. Carino, M. B. Cohen, and J. F. Koener. 1995. Cytochrome P450 in the house fly:

Structure, catalytic activity and regulation of expression in an insecticide-resistant strain. Pestic. Sci. 43: 233–239.

Ffrench-Constant, R. H. 2013. The molecular genetics of insecticide resistance. Genetics 194: 807–815.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge, United Kingdom.

Grant, D. F., and B. D. Hammock. 1992. Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. Mol. Gen. Genet. 234: 169–176.

Hardstone, M.C., C. A. Leichter, J. G. Scott. 2009. Multiplicative interaction between the two major mechanisms of permethrin resistance, kdr and cytochrome P450-monooxygenase detoxification, in mosquitoes. J. Evol. Biol. 22: 416–423.

Harrewijn, P., A. Minks, and C. Mollema. 1994. Evolution of plant volatile production in insect-plant relationships. Chemoecology 6: 55–73.

Hemingway, J., and H. Ranson. 2000. Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45: 371–379.

Isman, M. 2000. Plant essential oils for pest and disease management. Crop Prot. 19: 603–608.

Isman, M., and C. M. Machial. 2006. Pesticides based on plant essential oils: From traditional practice to commercialization, pp. 29–44. *In* Rai, and Carpinella (eds.), Naturally occurring bioactive compounds. Elsevier B.V., Amsterdam, Netherlands.

Lopes-Lutz, D., D. Alviano, C. S. Alviano, and P. P. Kolodzieczyk. 2008. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry 69: 1732–1738.

Lee, S., C. J. Peterson, and J. R. Coats. 2003. Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored Prod. Res. 39: 77–85.

Maia, F. M. and S. J. Moore. 2011. Plant-based insect repellents: A review of their efficacy, development and testing. Malaria J. 10: S11

McLaughlin, G. A. 1973. History of pyrethrum, pp. 3–15. *In* J. E. Casida (ed.), Pyrethrum–the natural insecticide. Academic, New York, NY.

Moore, S. J., A. Lenglet, and N. Hill. 2007. Plant-based insect repellents. *In* M. Debboun, S. P. Frances, and D. Strickman (eds.), Insect repellents, vol. 14. CRC Press. Boca Raton, FL.

Oppenoorth, F.J. 1984. Biochemistry of insecticide resistance. Pestic. Biochem. Physiol. 22: 187–193.

Paluch, G., J. Grodnitzky, L. Bartholomay, and J. R. Coats. 2009. Quantitative structure-activity relationship of botanical sesquiterpenes: Spatial and contact repellency to the yellow fever mosquito, *Aedes aegypti*. J. Agric. Food Chem. 57: 7618–7625.

Pampana, E. J., and P. F. Russell. 1955. Malaria—a world problem. WHO Chron. Geneva, Page 4.

Pampana, E. J. 1963. A textbook of malaria eradication. Oxford University Press, London, England.

Poelman, E. H., M. Bruinsma, F. Zhu, B. T. Weldegergis, A. E. Boursault, Y. Jongema, and J.J.A. Loon. 2012. Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. PLoS ONE. 10: 1–13.

Porter, N. G., P. E. Smale, M. A. Nelson, A. J. Hay, J. W. Klink, and C. M. Dean. 2010. Variability in essential oil chemistry and plant morphology within a Leptospermum scoparium population. N. Z. J. Bot. 36: 125–133.

Ranson, H., R. N'Guessan, J. Lines, N. Moiroux, Z. Nkuni, and V. Corbel. 2011. Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? Trends Parasitol. 27: 91–98.

Ruigt, G.S.F. 1985. Pyrethroids, pp. 183–261. *In* G. A. Kerkut, and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 12, Pergamon, Oxford.

Sangwan, N. S., A.H.A. Farooqi, F. Shabih, and R. S. Sangwan. 2001. Regulation of essential oil production. Plant Growth Regul. 34: 3–21.

Santolamazza, F., M. Calzetta, J. Etang, E. Barrese, I. Dia, A. Caccone, M. J. Donnelly, V. Petrarca, F. Simard, J. Pinto, et al. 2008. Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. Malaria J. 7: 74.

Service, M. 2012. Introduction to mosquitoes (Culicidae), pp. 2–34. *In* Medical entomology for students, 5th ed. Cambridge University Press, New York, NY.

Stamopoulos, D. C., P. Damos, and G. Karagianidou. 2007. Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 43: 571–577.

Tattersfield, F., R. P. Hobson, and C. T. Gimingham. 1929. Pyrethrin I and II: Their insecticidal value and estimation in pyrethrum (Chrysanthemum cinerariaefolium). J. Agric. Sci. 19: 266–296.

Tong, F., and J. R. Coats. 2010. Effects of monoterpenoid insecticides on [^{3}H]-TBOB binding in house fly GABA receptor and $^{36}Cl^{-}$ uptake in American cockroach ventral nerve cord. Pest Biochem. Physiol. 98: 317–324.

Tong, F., A. D. Gross, M. C. Dolan, and J. R. Coats. 2012. The phenolic monoterpenoid carvacrol inhibitis the binding of nicotine to the housefly nicotinic acetylcholine receptor. Pest Manage. Sci. 69: 775–780.

Vallejo, I., L. Rebordinos, I. G. Collado, J. M., Cantoral and Fernandez. 2001. Differential behavior of mycelial growth of several botrytis cinerea strains on either patchoulol- or globulol-amended media. J. Phytopath. 149: 113–118.

(WHO) Insecticide resistance and vector control. 1970. Seventeenth report of the WHO Expert Committee on Insecticides. WHO Technical Report Series no. 443. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 2013. Foreword. World Malaria Report. WHO, Geneva.

(WHOPES) World Health Organization Pesticide Evaluation Scheme. 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Control of neglected tropical diseases: WHO Pesticide Evaluation Scheme, Geneva.

Yanagawa, A., F. Yokohari, and S. Shimizu. 2010. Influence of fungal odor on grooming behavior of the termite, *Coptotermes formosanus*. J. Insect Sci. 10: 141.

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